PLATE 3.

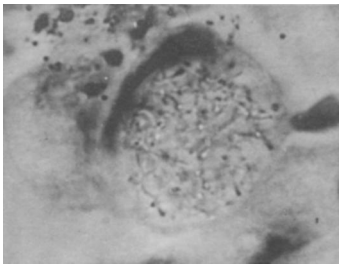

FIG. 1.

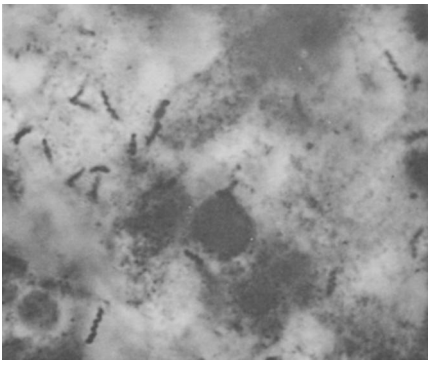

FIG. 2.

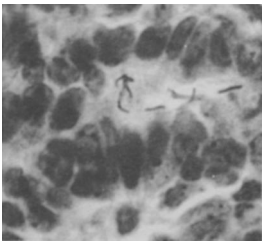

FIG. 3.

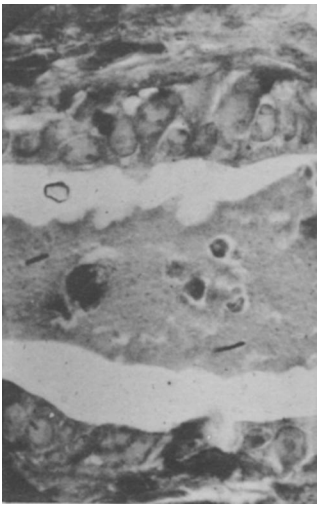

FIG. 4.

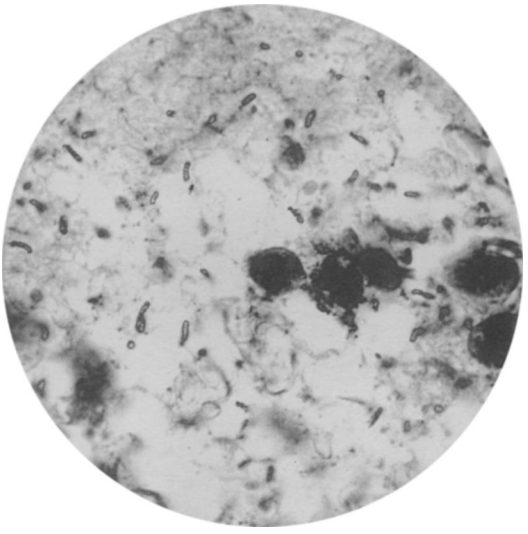

FIG. 5.

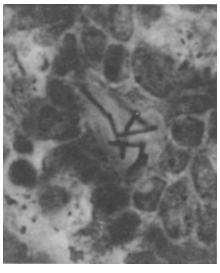

FIG. 6.

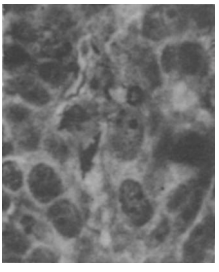

FIG. 7.

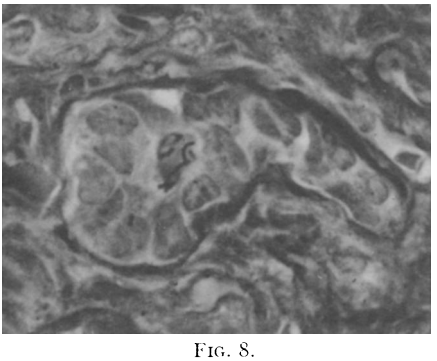

FIG. 8.

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# A SPIROCHETE IN PRIMARY AND TRANSPLANTED CARCINOMA OF THE BREAST IN MICE.*

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IN the early spring of 1905 the writer first observed in the epithelium of a retrogading transplanted tumor of the Jensen series, certain curious structures which were the subject of a publication by G. N. Calkins and G. H. A. Clowes under the title "Some Artefacts in Mouse Carcinoma."[1] The material in which the structures in question were first seen was hardened in an excess of mercury and contained, besides the structures about to be specified, curious deposits in crystalline form, both intra- and extra-cellular. The structures which attracted our attention at that time were very fine filaments of varying length, frequently possessed of clubbed ends, located in the protoplasm. They are illustrated by Figs. 5 and 6 of Plate 17, and by drawings by Calkins in Figs. 6, 7, 8, 9, and 10 of Plate 18.

The first impression which we had of these structures was that they must be some sort of parasitic form as yet unrecognized, but our inability to distinguish them from the deposits of mercury in the surrounding tissues, and in the nuclei and protoplasm of the cells of the

* Received for publication, March 12, 1907.

[1] *Jour. Infect. Dis.*, 1905, 2, p. 555.

tumor, led to the conclusion by Calkins and Clowes that they were artefacts, and the specimen was thereupon described and published.

So deeply impressed was the writer that certain of these forms must be parasitic that he never abandoned that point of view; and that Clowes and Calkins were also of the opinion that the bodies represented unusual structures impregnated with mercury is shown by the following sentence in their publication, on p. 559: "While these deposits are to be interpreted as artefacts, the fact must not be overlooked that something of an unusual nature is present in these cancer cells and tissues, upon which the salts of the fixing agent work in forming the deposits of various kinds."

Being deeply impressed with the possible significance of these curious filamentous structures in the vacuoles of the cells of the transplanted mouse tumor, and having made various attempts to find a method which would better demonstrate them if present, the writer at once saw, on the publication of Levaditi's article,[1] a close resemblance between these filamentous structures and the involution forms of *Spirochaeta pallida* so frequently encountered in syphilitic tissue. Thereupon a systematic investigation of all the transplanted mouse tumors in the State Cancer Laboratory was undertaken by the Levaditi method. The results of this systematic examination are here reported.*

An examination of transplanted tumors from three separate strains which are at present in existence in this laboratory has shown the constant occurrence of a characteristic organism in the transplanted tumors of each. The tumors from which these strains are derived are first the famous Jensen, which is now, in our hands, in its twentieth generation; a very virulent tumor known as the Brooklyn tumor, in its twentieth generation; and a less virulent tumor, known as the Springfield tumor, in its twelfth generation. All three of these tumors are carcinomata of the breast. They present practically identical characteristics, being carcinoma solidum, with occasionally adenomatous types.

[1] *Ann. de l'Inst. Pasteur*, 1906, 20, p. 41.

*The title of this paper was on the program, but it was not read, at the meeting of the British Medical Association in Toronto, August 22, 1906. The facts in substance were presented at a meeting of the Academy of Medicine in Cleveland, January 11, 1907, and before the State Medical Society of New York at Albany, January 29, 1907.

The tumor known as $3^{10}$, in which the original threadlike structures impregnated with mercury were discovered in March, 1905, belongs in the second generation of the Jensen strain. We have examined by the Levaditi method 914 E, 914 G, 915 A, and 915 D, out of the fourteenth generation of this tumor. All of these transplanted tumors were movable beneath the skin and uncontaminated. At the margin of the tumors in the Levaditi sections is found in the immediate neighborhood of the infiltrating edge of epithelium, a small spiral organism from 2.5 to 7.8 $\mu$ in length, with from four to as high as 13 or 16 nodes or corkscrews. The organism is 0.6 $\mu$ in average width; the ends are slightly rounded, and there is no evidence of an undulating membrane or flagellum.

Of the Springfield tumor 900 F, 921 F, 921 H, from the sixth generation, were subjected to the Levaditi method. An organism was found at the margin which was identical in appearance with that found in the Jensen tumors, bore the same relation to the epithelium, and was distributed in the same manner.

Of the Brooklyn tumor 741 A from the ninth generation, 763 E eleventh generation, 918 G thirteenth generation, 904 K thirteenth generation, 935 H fourteenth generation, 918 C fourteenth generation, 1039 E fifteenth generation, 1088 C sixteenth generation, 1113 A seventeenth generation, were examined by the Levaditi method. In every section of every one of these tumors the same characteristic spirochetes were found as described in the Jensen and Springfield tumors. These were in great numbers about the margin of the tumor in the connective tissue immediately adjacent to the epithelium. The organism is also occasionally found in the stroma or between the epithelial cells through the substance of the tumor, but the characteristic distribution is in the zone of round-celled infiltration and between the epithelial cells at the infiltrating edge of the tumor. Some of the larger of these tumors showed in their centers the usual areas of necrosis. A careful examination of these areas of necrosis failed to show the presence of any microorganisms of any kind.

Having determined the practically constant occurrence of this organism by the Levaditi method, attempts were made to stain it by Giemsa, by the various flagella stains, or by Wright's method, all

without success, although the material which was used was known to contain large numbers of organisms. The organism could, however, be easily detected in fresh material. It is rather difficult to see because of its low refractive index and the high refractive index of the fluid portions of the tumor. Patient search has, however, in every instance in which fresh material has been examined, demonstrated well-defined, actively motile organisms. Measurements made of these fresh, living organisms correspond with those made of the hardened organism in the Levaditi sections. Dr. F. G. Novy, who examined for me a fresh preparation from an uncontaminated mouse tumor, made the following measurements: From 2.5 to 7.8 $\mu$ in length and from 4 to 13 turns.

The proper position of this organism and a more definite description of its characteristics have been kindly undertaken by Professor Calkins.

Having determined the constant occurrence of a living spirochete in all of our transplanted mouse tumors, it was of the greatest importance to discover with what frequency they could be found in unattached, uncontaminated primary mouse carcinomata. We have up to the present time examined 10 primary mouse carcinomata. One of these was badly impregnated, so that it was impossible to determine the structure of the tumor satisfactorily, and it was therefore excluded. Of the remaining nine, the organisms have been found in all. They were found with no difficulty whatsoever in five. These are known as G-7, G-E, G-A, G-C, Springfield No. 8, and Buffalo I. In the tumors known as G-10 and G-8 the impregnation was imperfect and the organisms were of a pale brown, but could, however, be definitely distinguished. Tumor G-D was large and on its superior aspect slightly attached to the skin. The greater part of the central portion of the tumor was necrotic; only a very narrow margin at the periphery of the deeper portions of the tumor was in a state of active growth, and in these regions the organisms, although few in number, could be easily found.

It is to be noted that all of these tumors, with the exception of G-D and Buffalo I, were freely movable beneath the skin and small. All the G tumors were obtained at different times from a dealer in Massachusetts, whose animals are frequently affected with carcinoma of

the breast. We have received from this dealer, in the course of the last eight months, 31 tumors. The tumor designated Springfield 8 was a small primary tumor obtained from a dealer in Springfield, Ohio, who has furnished us with three tumors in the last year and a half. It is from this dealer that we have obtained a cage from which in the course of three years have been taken over sixty mice with carcinoma of the breast. The cage with its contents was brought to Buffalo, and since its arrival at this laboratory five mice with cancer of the breast have been removed from it. Both of these series of tumor mice have been previously reported.[1] The Buffalo I tumor was found in a cage of pet mice in the home of a Buffalo physician. To these must be added a primary cancer of the breast found by Calkins in a mouse in his laboratory at Columbia University, New York, which contains the organism.

For control of the work up to this point we have impregnated and sectioned pieces from all the organs and subcutaneous tissues of five supposedly normal mice. A prolonged search of all these tissues has given negative results, but more extensive controls will be necessary before the distribution of this organism, which obviously must be very wide, can be defined.

Before passing to a study of the relation of these organisms to the structure of the tumors in which they occur, it may be well to review the scanty literature on the presence of spirochetes in mouse and other carcinomata.

The first reference to the presence of spirochetes in malignant tumors is found in the report of Hoffman.[2] Under Hoffman's direction Mulzer found spirochetes in the scrapings from a case of carcinoma of the cervix and in two squamous epitheliomata, one from the face and the other from the abdominal aspect. All of these tumors were *advanced and ulcerated.* In all three cases the spirochetes showed coarse gyrations and stained more deeply than *Sp. pallida.* Certain individuals were encountered, however, which did not stain so deeply, and in the size and number of their gyrations closely approximated the latter organism.

Löwenthal[3] also describes the detection of spirochetes by the Giemsa method, on the surface of ulcerated tumors. He calls attention to the fact that they are not only present in human tumors under such conditions, but that he has found them in numbers associated with the usual bacteria on the ulcerated surface of a tumor in the neck of a dog. He calls attention to the similarity of the organism he observed to a small spirochete found in feces. This organism usually stains a pale blue with

[1] *Jour. Amer. Med. Assoc.*, 1907, 48, p. 15.

[2] *Berl. klin. Wchnschr.*, 1905, 42, p. 880.

[3] *Ibid.*, 1906, 43, p. 283.

Giemsa, and stains also with a borax-methylene blue solution which does not stain *Sp. pallida*. The organisms are from 2.5 to 6 $\mu$ in length. He could not determine their thickness, but estimated it to be from 0.25 to 0.5 $\mu$. They appear plumper when stained with Löffler's flagella stain. The gyrations are very close together and abrupt. The organism has usually from 4 to 12 corkscrews, and he estimates these to be about 0.5 $\mu$ apart. He proposes as the name for this organism "*Spirochaeta microgyrata.*" Up to the present he has been unable to determine the presence of flagella, and an undulating membrane was not visible, but he inferred from the increased plumpness of the organism, when stained with Löffler's flagella stain, that one was probably present. The long examples of the organism were frequently found to consist of two individuals attached at the ends. He considers that it may be readily differentiated from *Sp. pallida*. Besides this organism he frequently found a larger form from 5 to 11 $\mu$ in length with corkscrews from 1.5 to 2 $\mu$ apart which stains blue with Giemsa. In all cases where Löwenthal detected the spirochetes on the ulcerated surface of tumors he found regularly rodlike, straight or slightly bent, sausage-like structures which in their appearance and form closely resembled the so-called fusiform bacillus which accompanies the spirochetes, known as *Spirillum sputigenum*, so frequently found in the buccal cavity. Several authors have already advanced the view that there is a genetic relation between these fusiform structures and the spirochetes with which they are associated. Löwenthal advocates the same view in connection with the fusiform structures accompanying the small spirochetes found on the surface of ulcerating tumors, for the reason that they invariably accompany these organisms. He states that wherever he found fusiform structures in a smear, on further search he never failed to find the small spirochetes in question.

Borrel[1] found helminthia in two inclosed mouse tumors surrounded by large numbers of leucocytes and endothelial phagocytes. The worms were transported to the tumors through the blood vessels after penetrating the intestinal wall. In the neighborhood of these worms Borrel states that large numbers of spirochetes were present, and in a very cachectic mouse sent from Ehrlich's laboratory, in which the tumor was not ulcerated, he found large numbers of spirochetes. These had coarse gyrations. In the two cases described in Paris the spirochetes were of different form, very small, and with closely packed spirals. The tumors thus examined were movable beneath the skin, were not ulcerated, and at the time of publication were still living. He concluded that it was impossible to draw etiological conclusions from these observations, but he found it of great interest that spirochetes and worms were found in these particular strains.

Spirochetes in unulcerated human cancer, demonstrated by the silver method, have as yet been reported by but one observer, Friedenthal.[2] Friedenthal is of the opinion that the structures, which are clearly spirochetes from his illustrations are not organisms, but represent condensations of the protoplasm impregnated with silver. He obviously published his observations to show that so-called spirochetes of syphilis which Schultze and others hold to be nerve fibers and elastic fibers impregnated by the silver method, are not necessarily all attributable to the misinterpretation. Inasmuch as the structures he described were within the epithelial cells of the tumor, Friedenthal held that some of them were condensations of the protoplasm, etc., and not nerve endings or elastic fibers.

[1] *Comp. rend. de la Soc. de Biol.*, 1905, 58, p. 770.

[2] *Berl. klin. Wchnschr.*, 1906, 43, p. 283.

It will be seen that of these observers Mulzer describes a coarse spirochete on the ulcerated surface of human tumors; Löwenthal describes a coarse and a smaller organism, known as *Sp. microgyrata*, on the surface of ulcerated human tumor and in a tumor in the neck of a dog. None of these authors attributes any significance to the presence of these organisms, as they were found under conditions which could not justify any suggestion that they were other than accidental in their occurrence. Borrel found in two cases a small spirochete in connection with helminthia and an organism with coarse gyrations in a cachectic mouse tumor sent from Ehrlich's laboratory. Borrel does not state whether these tumors were primary or transplanted tumors, but the inference is that they were transplanted. He believed that the organisms were conveyed to the tumors by the helminthia in the first two cases, and drew no conclusions as to their possible etiological significance in any of the three cases described.

Besides spirochetes associated with mouse tumors, Wenyon[1] describes a spirochete which he found in the blood of a gray mouse in the Pasteur Institute. From this mouse he succeeded in infecting other mice; in these both the blood and the spleen contained the organism and could be used for further inoculation. He inoculated as many as fifty mice and never encountered a case of natural immunity. The organisms stained readily with any of the ordinary stains—Giemsa, fuchsin, methylene blue, etc.

"In stained preparations the spirochete is seen as a uniformly staining spiral; the longer forms, however, show a clear unstained central spot (see Diagrams Nos. 8 and 9). The ends are slightly tapering; there is no sign of a nucleus or undulating membrane. As just mentioned, the larger forms have a clear spot at their center, and in some of these the body of the spiral tapers toward this spot. In some cases two small spirals are attached end to end by an unstained region. These forms are evidently stages of transverse division. No indications of longitudinal division were seen nor any mode of reproduction other than the one just mentioned.

"The number of the turns of the spiral varies from six in the longest forms to two in the shortest. The lengths of the spirochetes vary from 6 or 7 $\mu$ to 3 or 4 $\mu$. The width is about 2 $\mu$.

"The spirochete was always seen in the spiral form; no other forms were found at any time."

Wenyon states that, after discussing the matter with M. Borrel, they arrived at the conclusion that the organism just described and the one observed by Borrel[2] in the juice of malignant growths of mice are the same organism; comparison of Borrel's preparations and those of Wenyon lead to the same conclusion. Wenyon attempted to test the theory of Borrel that these organisms had migrated into the tumors from the intestinal tract, by inoculating mice with mucus from the intestinal tract of mice which contained large numbers of varying kinds of spirochetes. These conclusions were negative; no infection or abscess resulted. The mucus from the intestinal tract of a mouse infected with *Spirochaeta m.* likewise gave negative results. Wenyon concluded that there is no evidence that these spirochetes have originated in the intestine.

The description given by Wenyon of the organism found in the blood of otherwise normal mice clearly distinguishes it from the organism which we have described. If it be identical with the organism found by Borrel in his mouse tumors that would seem

[1] *Jour. Hyg.*, 1906, 6, p. 580.

[2] *Compt. rend. de la Soc. de Biol.*, 1905, 58, p. 770.

to distinguish this organism from the organism which we have found. Borrel does not give the method by which he succeeded in demonstrating the organism found in his mouse tumors, but as his publication antedates that of Levaditi, it is highly probable that it was by means of the Giemsa or some aniline staining method. Whether our inability to stain our organism with any of the aniline methods will prove a means of distinguishing it from other small organisms, such as that of Löwenthal and the smaller organisms found by Borrel in combination with helminthia, remains to be seen, but the forms with the coarser gyrations appear to be clearly distinguishable from our organism.

The distribution of the organisms through the primary tumors examined is of great importance. A careful study of these tumors will appear to throw some light on whether or not the organisms were accidental in their relation to the tumors in which they are found, or whether they may bear some etiological relation. A description of one of these tumors will suffice for all, the only difference being in the number of organisms which the small fragments impregnated with silver happen to contain. The greatest number were found in the primary tumor known as G-7, and, with the qualification that the other tumors contain fewer organisms, the description of this tumor will apply to the remaining eight.

This tumor was sent from Massachusetts and remained in the laboratory about two days. The tumor was about the size of a small bean, measuring approximately 1 cm. by 0.6 cm. in its greatest diameter. It was slightly flattened, freely movable beneath the skin, and was situated on the ventral aspect. The mouse was killed and the tumor removed September 20, 1906. Weight of mouse 33.5 grams, weight of tumor 1.5 grams. Six mice, Lot 1031, were inoculated with portions of the tumor. Results to date negative. Small fragments of the tumor were hardened in formalin, and some of them impregnated by the Levaditi method.

An examination of a section of the tumor hardened in formalin and stained with Borrel's method, with low power, shows the tumor to be a rapidly growing adenocarcinoma of the breast. In many portions the tumor assumes the characteristics of a rapidly growing carcinoma solidum. At the edge of the tumor is the usual characteristic proliferation of the connective tissue invaded by prolongations and nests of epithelium from the tumor mass. In some portions of the periphery a more or less definite connective tissue capsule is present. Scattered through the tumor are the small areas of necrosis which characterize rapidly growing tumors of this type. The stroma is in some portions of the tumor poorly developed, in others well defined. Here and there, especially at the margins where the tumor is penetrating into the connective tissue structure, the tendency to the adenomatous type is quite marked. In these

regions cysts of considerable size can be found, filled with the usual coagulated fluid mixed with cell detritus. A careful examination of such a section with oil immersion fails to show the presence of any organisms, even after comparison with sections from the immediate neighborhood impregnated with the Levaditi method in which plentiful spirochetes are present. Not even unstained structures which one might identify can be determined.

Sections from small pieces of the tumor impregnated by the Levaditi method, examined under high power, present a very striking appearance. First of all, lying free within the cysts in the coagulated contents, are found sharply defined, intensely black spirals, measuring from 2.5 to 7.8 $\mu$ in length. The corkscrews are from three or four to 13 or even more in number. These organisms are more or less regularly distributed through the cyst spaces (see Fig. 4, Plate 3), and present so characteristic an appearance that they can, to our minds, never be confused with nerve endings. In the rapidly growing portions of the tumor between the epithelial cells one frequently encounters aggregations of five or six, or even a dozen, of these characteristic organisms lying in small, clear spaces between the epithelial cells, as though they were surrounded by some clear fluid producing a form of vacuole (Fig. 6, Plate 3).

Passing to the margins of the tumor, one finds that in the nests of epithelium penetrating into the connective tissue stroma the lumena of the small tubular structures frequently contain aggregations of organisms, and here and there free epithelial cells with organisms in their protoplasm. One can easily imagine that the development of the larger cysts in the tumor might be determined by the proliferation of the epithelium around the small groups of organisms thus found. Beginning with a secretion of fluid around the organisms, as shown in Fig. 6, Plate 3, the proliferation of the epithelium might produce the beginning of a cyst, such as is represented by Figs. 8 and 3; and the continued secretion of fluid and associated proliferation might ultimately terminate in the development of a larger cyst, such as Fig. 4. The invariable presence of organisms in the larger cyst cavities would suggest such a possibility. The organisms are distributed in this tumor in such a way that the largest numbers of organisms are in the most rapidly growing portions of the periphery. Occasional organisms are found in the connective tissue beyond the growing edge of the tumor. This is especially well seen where the epithelium is penetrating into the adjacent fat tissue. Here organisms will be found between the fat cells a short distance in advance of the epithelium, but when one investigates the surrounding normal tissues beyond this zone, no organisms are to be found. Where the stroma of the tumor is best developed one frequently finds organisms in the stroma. Where this is not so well defined, they lie between the epithelial cells, as shown in Figs. 3, 6, and 8, Plate 3. Occasionally organisms can be found within the epithelial cells. Here they are frequently curved into ring forms (Fig. 7, Plate 3), or in aggregations suggesting agglutination (Fig. 7, Plate 3). The characteristic spiral form of the organism is not always apparent. Occasional forms will be met where the silver appears to have impregnated the organisms homogeneously. This is usually the case with organisms within the protoplasm of epithelial cells (Figs. 7 and 8, Plate 3), and in these cases the organism frequently presents a beaded appearance suggestive of involution forms or degeneration approaching disintegration. In regions where phagocytosis is active one will find fragments of the organisms in the protoplasm of the epithelium, the organisms in these cases appearing to break up into minute granules or short S-shaped structures

similar to the changes in *Sp. Obermeieri* in mononuclear macrophages in the peritoneal cavities of hyperimmunized rats (Pfeiffer's phenomenon).

The examination of sections of the remaining eight carcinomata of the breast in mice differ in no way from the findings in G-7. In the tumors in which there are few organisms these are found exclusively at the very margin of the growing edge, in the center of cell nests, and in the connective tissue stroma surrounding the tumor.

In the transplanted tumors the distribution is even more pronouncedly at the margin of the tumors. In rapidly growing, early transplants, the organisms are massed in the zone of connective tissue proliferation at the margin, and in the more virulent tumors, such as the Brooklyn tumor, the organisms are frequently present in great numbers (Fig. 5, Plate 3). Involution forms and disintegrating organisms are very frequent in these areas. Phagocytosis does not seem to be so active in these tumors. Careful examination of the sections from the Springfield, Jensen, and Brooklyn strains shows no differences in appearance or measurements, distribution, or characteristics of the organisms in the different strains. We appear here to be dealing with a definite organism. In large, late transplanted tumors, in which there are areas of extensive necrosis, the organisms are exclusively confined to the growing margins of the tumor. When, as occasionally happens, the necrosis extends to the periphery of the tumor, the adjacent connective tissue zone contains either no organisms or occasionally disintegrating or involution forms. In two large tumors examined, which were badly contaminated with bacteria, no spirochetes whatever could be found.

With the determination of the practically constant association of this characteristic organism in primary and transplanted mouse tumors naturally arises the question as to their significance. Although it will require a prolonged and careful search through other types of tumors, especially in other animals and in human beings, before the relation of this organism to the tumors in question can be definitely ascertained, still it would seem not improper to discuss in the light of the striking evidence of cage infection in this very group of tumors, the possible etiological significance of these organisms. Up to the present time those who have sought for parasites in cancer have searched for an intracellular organism, and at first glance it would

seem difficult to understand how an organism with the distribution of the one in question could be responsible for the epithelial proliferation necessary for the production of a malignant growth. We have for some time assumed that exrtacellular organisms might be the cause of the proliferation of epithelium through the medium of some toxic substance which they elaborate. Evidence of the existence of some bio-chemical substance of this sort in tumors has come to us in the course of our experimentation. The interesting work of Clowes and Baeslack from this laboratory on "The Influence Exerted on the Virulence of Carcinoma in Mice by Subjecting the Tumor Material to Incubation Previous to Inoculation," is a case in point, Clowes having reasoned that the best explanation of the marked increase in virulence produced by a short period of incubation resulted from the effect of increased temperature upon the rate of reaction of some stimulating substance contained in the tumors. These interesting observations have had a very positive and new light shed upon them by the work of Bernhard Fischer.[1] Fischer has made the discovery that there are certain chemical substances (thus far the fat stains, scarlet R, Sudan III, and indophenol) which possess a positive chemotactic quality for the epithelium of the skin in rabbits, and possibly some other animals. This chemotactic or attractive quality is exerted only when these fat stains, dissolved in olive oil, are injected into the subcutaneous tissue beneath the skin. Here they set up a chronic connective tissue proliferation similar to that found about the margins of beginning epitheliomata of the skin. The scarlet R oil penetrates into all the lymph spaces and crevices of the tissue and, with the advent of the chronic connective tissue proliferation, exerts a chemotactic attraction for the epithelium of the deeper layers of the adjacent skin. Oil alone does not exert an activity to this extent, and it is Fischer's opinion that part of the scarlet R, although but slightly soluble in water, is taken up by the lymph and transplanted to the epithelium which it stimulates to proliferation. Within a short period (three weeks) the presence of the scarlet R in the subcutaneous tissue produces an active proliferation of the epithelium of the skin, of the hair follicles and sebacious glands, associated with the presence of typical and atypical karyokinetic figures. The epithelium pene-

[1] *Münch. med. Wchnschr.*, 1906, 53, p. 2041.

trates into the surrounding connective tissue in the form of charac teristic prolongations and nests, such as characterize the beginning of squamous epithelioma of the skin. Typical epithelial pearls are formed, and Fischer states that at this stage of the process the histological appearance is indistinguishable from epithelioma of the skin. The illustrations which he gives confirm this opinion. That it is the scarlet R which attracts the epithelium is shown by the epithelium growing down to, surrounding, and gradually removing the saturated oil drops. Where the scarlet oil penetrates into the lymph spaces the epithelium proliferates in the lymph spaces in pursuit of it, and in one case, where Fischer wounded the cartilage with the needle used for injection, he found the epithelium penetrating into the lymph spaces of the cartilage and the clefts which contained the scarlet oil. That part of the coloring matter is diffused through the tissues; and that it is in this way that scarlet R produces its first effect upon the more or less distant epithelium is shown by the occasional extensive staining of the fat constituents of the adjacent cartilage cells where the scarlet R has been injected into the connective tissue in the immediate neighborhood. Fischer found that scarlet R affects only the epithelium of the epidermis of the rabbit. Attempts to produce similar proliferations by injecting the stain into the breast, under the epithelium of the stomach and intestinal tract, have proven invariably negative. In one case of a dog in which a large amount of scarlet R was injected beneath the skin producing the characteristic proliferation of the epithelium, small nodules apparently derived from proliferation of the alveolar epithelium of the lung were found, and Fischer suggests that possibly this type of epithelium may prove less specific than others and also react to the stimulus of scarlet R.

From his experiments it would appear that scarlet R is capable of affecting only one kind of epithelium, the epidermis. Upon the cells of this structure it exerts, from its position in the subcutaneous tissue, an attractive or chemotactic function which causes the epithelium to proliferate in the deeper structures, producing in the height of its activity, a picture indistinguishable from beginning carcinoma. Here, however, the analogy ceases; for when the scarlet R is entirely absorbed by the epithelium, the cells rapidly hornify,

and the entire process subsides. Fischer points out that all that is needed to extend this process into carcinoma would be the local, continuous production of some chemical substance similar in its affinities and characteristics to scarlet R. In a footnote he states that he appreciates that the advocates of the parasitic theory can utilize his observations in support of some parasite working through the medium of an *attraxine*, as he calls this substance; in which case it would be necessary, however, to assume a special organism for every type of epithelium subject to cancerous transformation.

The distribution of the organism which we have described corresponds in very striking degree to the distribution of the scarlet R in Fischer's experiments. The absorption and removal of the scarlet oil by the epithelium finds its counterpart in the evidence of phagocytosis which we have noted. If the spiral organisms found in our tumors are the cause of these tumors, then they produce the proliferation of the epithelium through the medium of some toxic substance which they elaborate. That such a toxic substance possibly exists has, as we have pointed out, already been shown by Clowes. To our minds, the necessity of assuming a different organism for each type of epithelium rather simplifies than complicates the problem, and it is not impossible that organisms belonging in the same class, or of widely different characteristics, may possess the power of elaborating specific toxic substances. A striking possibility in this connection is the so-called Bilharzia disease, in which typical cancer of the bladder is associated with the presence of the embryos of the worm in the bladder wall. It is also of interest that in Bilharzia disease no metastases have ever been found, although the local disease of the bladder presents the characteristics of infiltrating carcinoma.

The evidence of immunity associated with these tumors is, to our minds, likewise suggestive of a possible etiological significance of these organisms, and, in this connection, the conditions found in the tumor 3[10] referred to at the beginning of this article are of great interest. This tumor at the time of its removal and hardening had begun to retrograde and presented the histological characteristics of spontaneously retrograding tumors. It is possible that this tumor was hardened at the moment when a very active phagocytosis on the part of the remaining epithelium was in progress. As shown in Fig. 1,

Plate 3 of this article, and in Figs. 5 and 6, Plate 17 of Calkins and Clowes's article in this journal, many of the epithelial cells of this tumor contained large numbers of small, rodlike structures, which, in the writer's opinion, are the organisms we have described, incrusted with mercury. If one studies the vacuoles containing these structures in sections of this tumor, one can trace through the smaller vacuoles a gradual disintegration and final disappearance of these structures within the vacuoles. The whole presents a picture which strongly suggests the description given by Novy and Knapp[1] of the disintegration of *Sp. Obermeieri* in the bodies of phagocytes after the injection of blood containing large numbers of this organism into the peritoneal cavity of recovered rats. As the entire process in the case of *Sp. Obermeieri* occurred in a period of less than 10 minutes, it would appear that, if the appearance found in this tumor illustrated such a phenomenon in the mouse tumors, the tumor was placed in the hardening agent at the psychological moment. It will, however, require further experimentation to confirm the significance of these appearances.

It is obviously too early to draw far-reaching conclusions from the presence of this organism in our mouse tumors. First of all, more extensive experiments must be carried out. These are in progress, and the organism is being studied in all its relations to the tumor in question, in which investigations Drs. Calkins and Clowes have joined. The description of what appear to be similar organisms impregnated with silver in a case of uncontaminated human cancer of the breast by Friedenthal naturally suggests that a further search for these organisms in human tumors may lead to positive results in this connection, and such a systematic search has already been inaugurated. At present it appears to us that the constancy with which this organism is found in the primary and transplanted mouse tumors is very striking; that in the light of Fischer's work it would appear to us quite possible that an extracellular organism distributed as this organism is distributed, could be the cause of the proliferation; that the evidence of phagocytosis and the detection of these organisms at times within the protoplasm of epithelial cells would sufficiently explain the establishment of metastases by the transportation of

[1] *Jour. Infect. Dis.*, 1906, 3, p. 291.

infected cells. The organism should be found with continued regularity, in which case it would appear to bear the same relation to these tumors as does *Sp. pallida* to syphilis.

SUMMARY.

1. In 1905 there were found in vacuoles in the epithelial cells of a retrograding mouse tumor, fine, rodlike structures impregnated with mercury. These the writer held to be parasites.

2. Attempts to fix these structures were not successful until the advent of Levaditi's method. With this method a characteristic spiral organism 2.5 to 7.8 $\mu$ in length and 0.6 $\mu$ in width, with 4 to 13 turns per individual, has been demonstrated in 10 consecutive spontaneous carcinomata of the breast in mice obtained from Massachusetts, Ohio, and New York. In one other tumor the hardening method was a failure.

3. An examination of sixteen transplanted mouse tumors from three different sources shows the presence of the same organism in all tumors examined.

4. An examination of fresh materials from all transplanted uncontaminated tumors from these strains demonstrates the organism in the living state. It is frequently motile and is found with difficulty.

5. Measurements made on the fresh organism correspond closely with the measurements made in the stained preparations.

6. The distribution of the organism through the primary tumors shows that they are most prevalent in the most actively growing portions of the tumor; that they live in the connective tissue at the margin of the tumors, and in the stroma of the tumor; that they are found between the epithelial cells of the tumor and in the cyst cavities of the tumors where these are present.

7. In the early transplanted tumors the organisms are found in the connective tissue zone at the growing edge and between the cells at the growing edge.

8. The more virulent tumors contain the greatest number of organisms.

9. In both primary and transplanted mouse tumors evidences of phagocytosis on the part of the epithelial cells are to be found. The organism in these cells frequently assumes the form of rings and breaks down into small S-shaped segments and granules.

10. In two tumors badly contaminated by bacteria no spirochetes could be found.

11. The examination of organs and subcutaneous tissues of five normal mice by the Levaditi method has shown no spirochetes.

12. All attempts to stain the organism with aniline stains to date have proven unsuccessful.

13. The organism is morphologically distinguished from the organism described by Wenyon in the blood of mice.

14. It would appear to be distinguishable from the *Spirochaeta microgyrata* of Löwenthal and the organisms found by Borrel in mouse tumors, both of which are stained with the Giemsa stain, by its inability to take this stain.

15. Our observations do not as yet establish an etiological relation between this organism and cancer of the breast in mice, but the presence of the organism in primary mouse cancers with which it is regularly transplanted through many generations, greatly increasing in number as the tumors increase in virulence, instead of interfering with, and finally preventing, transplanation as do bacteria, is suggestive.

## DESCRIPTION OF PLATE 3.

FIG. 1.—Epithelial cell with large vacuole in protoplasm. Nucleus pushed to one side. Vacuole filled with fine rodlike bodies some of which show gyrations and beadlike structure. Taken from Jensen mouse tumor 1905. Sublimate fixation. Organism unstained. ×1,030.

FIG. 2.—Spirochetes from margin of transplanted Springfield tumor. Carcinoma solidum of the breast. Levaditi silver method. ×1,360.

FIG. 3.—Spirochetes between the epithelial cells of primary adenocarcinoma of the breast in mouse G-7. ×1,030.

FIG. 4.—Section through cyst in carcinoma of the breast, mouse G-7, showing three spirochetes in cyst contents. ×1,030.

FIG. 5.—Large numbers of spirochetes in margin of rapidly growing transplanted carcinoma of the breast, Brooklyn tumor. ×1,030.

FIG. 6.—Group of organisms between the epithelial cells of primary carcinoma, mouse G-7. ×1,030.

FIG. 7.—Spirochetes in protoplasm of epithelial cell, same tumor as Figs. 3, 4, and 6, showing phagocytic action of epithelium. Spirochetes in form of a ring and agglutinated organisms. ×1,030.

FIG. 8.—Spirochetes in protoplasm of ephithelial cell at the center of nest of growing epithelium. ×1,030.

In Figs. 1, 3, 4, 5, 7, and 8 the spirals are not easily seen, owing to the low magnification which was chosen to show the relation of the organisms to the tissue. In Figs. 2 and 6 the spirals are perfectly distinct. In every case, however, except in Figs. 7 and 8 the spirals are plainly discernible through the microscope. In Figs. 7 and 8, representing degeneration forms, the spirals are not intact.